PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference K2443-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/BE 03/00114	International filing date *(day/month/year)* 26.06.2003	Priority date *(day/month/year)* 26.06.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/22		
Applicant K.U. LEUVEN RESEARCH & DEVELOPMENT et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.01.2004	24.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Baminger, U Telephone No. +49 89 2399-2176

Form PCT/IPEA/409 (Cover Sheet) (January 2004)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE 03/00114

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-28 as originally filed

Claims, Numbers

1-57 filed with telefax on 15.07.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE 03/00114

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-57
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-57
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-57
	No:	Claims	

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/BE 03/00114

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-A-5486459
D2: EP-A-1138777
D3: US-A-5739004

5.1 The subject-matter of claim 1 is novel (Art. 33 (2) PCT) in view of the documents available to the examiner.

D1 (cf. ex. 1 and col. 14, lines 7-13) discloses a sterilization indicator comprising enzymes immobilized on a cellulosic disc (can be seen as a "first filler") and freeze dried. The disc is inserted into a vial and covered with mineral oil. A heat resistant sponge stopper is used to hold the mineral oil and disc in place in the vial.
D2 (cf. [40-42, 58, 62-63], ex. 1 and claims) discloses a temperature history indicator, particularly intended for the monitoring of storage conditions and the temperature range (thermal impact) of 0-80°C, preferably 5-65°C. Example 1 discloses an embodiment where the enzyme is dripped onto a non-woven fabric, freeze dried (solid, dehydrated mix) and incorporated into a polypropylene film - a blister packing type container - and a hermetic sealing barrier that prevents the entry of moisture into the container.
D3 (cf. ex. 12, ex. 1, col. 7, lines 19 - col. 8, line 42) discloses inter alia immobilised, absorbed, entrapped and encapsulated enzymes for the use in sterilisation indicators. The example 12 (that refers to ex. 1 for some features) discloses a vial with a dried enzyme/Sephadex mixture (solid dehydrated mix), covered by a Thinsulate (reg. TM) barrier.

The indicator devices of D1 and D3 differ in that they do not involve hermetically sealed containers. Both disclose semi-permeable barriers (mineral oil in D1 and Thinsulate (reg. TM) in D3. D1 and D3 also do not explicitly disclose a water content of the dehydrated mix (enzyme and filler) of below 0.6% by weight.

D2 (cf. [40-42, 58, 62-63], ex. 1 and claims) differs in that the water content of the dehydrated mix (enzyme and filler) is not given. Freeze drying (as in example 1) does not necessarily imply a water content of below 0.6% by weight.

5.2 For the purpose of examining the inventive step of claim 1, D1 can be regarded as the closest prior art.

The problem to be solved by the present application lies in the provision of an improved enzyme-based monitoring device for monitoring the thermal impact of thermal processing on an object under sterilisation or pasteurisation conditions.

The solution over D1 found in claim 1 of the present application is the use of a hermetically sealed container and a dehydrated mix containing the enzyme. The sealing should prevent the entry of moisture into the container so as to assure that the dehydrated mix does not exceed a moisture content of 0.6% by weight. This leads to an improved device - as described in the application on p. 17, lines 6-23.

There is no indication in the prior art for a person skilled in the art to modify D1 to arrive at the present invention. D2 discloses a hermetically sealed device, however the device is intended for monitoring storage conditions (i.e. less harsh heat conditions). The temperature range of use merely overlaps at the end point of 80°C, whereby the actual (preferred) working range in D2 is 5-65°C.

Therefore the subject-matter of claim 1 can be considered inventive according to Art. 33 (3) PCT.

5.3 The same applies mutatis mutandis to the subject-matter of claims 23 and 50.

CLAIMS

1. An enzyme-based monitoring device for monitoring the thermal impact of thermal processing on an object within a temperature range from 80°C to 160°C, said device comprising a container containing at least one enzyme and at least one barrier, characterized in that:
   - said container is a hermetically sealed container, and
   - said container encloses a solid dehydrated mix comprising said at least one enzyme and at least one first filler, wherein the water content of said dehydrated mix is below 0.6 by weight, hermetic sealing of the hermetically sealed container being obtained by means of said at least one barrier in order to prevent entry of moisture into said container .

2. An enzyme-based monitoring device according to claim 1, characterized in that said at least one enzyme represents between 0.001 and 10% by weight, of the solid dehydrated mix enclosed in said hermetically sealed container.

3. An enzyme-based monitoring device according to claim 1 or claim 2, characterized in that said at least one first filler represents between 90 and 99.999 % by weight of the solid dehydrated mix enclosed in said hermetically sealed container.

4. An enzyme-based monitoring device according to any of claims 1 to 3, characterized in that said at least one first filler is a non-porous filler.

5. An enzyme-based monitoring device according to any of claims 1 to 4, characterized in that said at least one first filler is an inorganic filler.

6. An enzyme-based monitoring device according to any of claims 1 to 5, characterized in that said at least one first filler is selected from the group consisting of glass beads, metal beads and silica beads.

7. An enzyme-based monitoring device according to any of claims 1 to 6, characterized in that said at least one first filler is an organic filler.

8. An enzyme-based monitoring device according to any of claims 1 to 7, characterized in that said at least one first filler consists of polymer beads.

9. An enzyme-based monitoring device according to any of claims 1 to 8, characterized in that said first filler consists of beads with an average size below about 0.3 mm.

10. An enzyme-based monitoring device according to any of claims 1 to 9, characterized in that said solid dehydrated mix further comprises at least one second filler.

11. An enzyme-based monitoring device according to claim 10, characterized in that said at least one second filler represents up to 10 %, preferably up to 5%, by weight of the solid dehydrated mix enclosed in said hermetically sealed container.

12. An enzyme-based monitoring device according to claim 10 or claim 11, characterized in that said at least one second filler is a water-soluble filler.

13. An enzyme-based monitoring device according to any of claims 10 to 12, characterized in that said at least one second filler is an organic filler.

14. An enzyme-based monitoring device according to any of claims 10 to 13, characterized in that said at least one second filler is selected from the group consisting of polyols and carbohydrates.

15. An enzyme-based monitoring device according to any of claims 10 to 12, characterized in that said at least one second filler is an inorganic filler.

16. An enzyme-based monitoring device according to claim 15, characterized in that said at least one second filler is selected from the group consisting of alkali and alkaline-earth metal salts.

17. An enzyme-based monitoring device according to any of claims 1 to 16, characterized in that said at least one enzyme is from bacterial, vegetal, animal or fungal origin.

18. An enzyme-based monitoring device according to any of claims 1 to 17, characterized in that said at least one enzyme is a bacterial α-amylase.

19. An enzyme-based monitoring device according to any of claims 1 to 17, characterized in that said at least one enzyme is a pectin methyl esterase.

20. An enzyme-based monitoring device according to any of claims 1 to 19, characterized in that the amount of said at least one enzyme in said device is below about 3 mg.

21. An enzyme-based monitoring device according to any of claims 1 to 20, characterized in that said hermetically sealed container is made from one or more moisture-impermeable materials selected from the group consisting of glass, silica, metals and polymers.

22. An enzyme-based monitoring device according to any of claims 1 to 21, characterized in that said hermetically sealed container is made from one or more layers.

23. Use of a solid dehydrated mix comprising at least one enzyme and at least one first filler, wherein the water content of said dehydrated mix is below 0.6% by weight, as a bio-integrator for monitoring the thermal processing of an object within a temperature range from 80°C to 160°C.

24. Use according to claim 23, wherein said object is in a particulate form.

25. Use according to claim 23 or claim 24, wherein said object is human or animal food.

26. Use according to claim 23, wherein said object is a medical tool or device.

27. Use according to claim 23, wherein said object is a pharmaceutical composition in the form of a liquid, syrup, cream or paste.

28. Use according to any of claims 23 to 27, wherein monitoring is based on residual enzymatic activity after said thermal processing.

29. Use according to any of claims 23 to 28, in the form of a process step in a pasteurization or sterilization process.

30. Use according to any of claims 23 to 29, wherein said at least one enzyme represents between 0.001 and 10% by weight, of said solid dehydrated mix.

31. Use according to any of claims 23 to 30, wherein said at least one first filler represents between 90 and 99.999 % by weight of said solid dehydrated mix.

32. Use according to any of claims 23 to 31, wherein said at least one first filler is a non-porous filler.

33. Use according to any of claims 23 to 32, wherein said at least one first filler is an inorganic filler.

34. Use according to any of claims 23 to 33, wherein said at least one first filler is selected from the group consisting of glass beads, metal beads and silica beads.

35. Use according to any of claims 23 to 32, wherein said at least one first filler is an organic filler.

36. Use according to any of claims 23 to 32, wherein said at least one first filler consists of polymer beads.

37. Use according to any of claims 23 to 36, wherein said first filler consists of beads with an average size below about 0.3 mm.

38. Use according to any of claims 23 to 37, wherein said solid dehydrated mix further comprises at least one second filler.

39. Use according to claim 38, wherein said at least one second filler represents up to 10 %, preferably up to 5%, by weight of said solid dehydrated mix.

40. Use according to claim 38 or claim 39, wherein said at least one second filler is a water-soluble filler.

41. Use according to any of claims 38 to 40, wherein said at least one second filler is an organic filler.

42. Use according to any of claims 38 to 41, wherein said at least one second filler is selected from the group consisting of polyols and carbohydrates.

43. Use according to any of claims 38 to 40, wherein said at least one second filler is an inorganic filler.

44. Use according to any of claims 38 to 40, wherein said at least one second filler is selected from the group consisting of alkali and alkaline-earth metal salts.

45. Use according to any of claims 23 to 44, wherein said at least one enzyme is from bacterial, vegetal, animal or fungal origin.

46. Use according to any of claims 23 to 45, wherein said at least one enzyme is a bacterial α-amylase.

47. Use according to any of claims 23 to 45, wherein said at least one enzyme is a pectin methyl esterase.

48. Use according to any of claims 23 to 47, wherein the amount of said at least one enzyme in the solid dehydrated mix is below about 3 mg.

49. Use according to any of claims 23 to 48, wherein said solid dehydrated mix is enclosed in a hermetically sealed container.

50. A method of monitoring the thermal impact of thermal processing on an object by means of an enzyme-based monitoring device, said device comprising a container containing at least one enzyme and at least one barrier, said method comprising the steps of:

(a) placing said enzyme-based monitoring device in contact with said object or in the neighbourhood of said object;

(b) exposing said object and said enzyme-based monitoring device to thermal processing at a temperature within a range from 80°C to 160°C for

sufficient time for degrading a substantial portion of said at least one enzyme without breaking said at least one barrier of said container;

(c) removing said container from contact with said object or from the neighbourhood of said object after completion of step (b);

characterised in that :

- said container is a hermetically sealed container,
- said container encloses a solid dehydrated mix comprising said at least one enzyme and at least one first filler, wherein the water content of said dehydrated mix is below 0.6% by weight, hermetic sealing of the hermetically sealed container being obtained by means of said at least one barrier in order to prevent entry of moisture into said container during thermal processing of said object, and
- said method further comprises the steps of:

(d) opening said hermetically sealed container and obtaining a sample of the at least one enzyme from said hermetically sealed container;

(e) measuring the residual activity of said at least one enzyme in the obtained sample, and

(f) using the measured residual activity as a means to quantify the thermal impact of the thermal processing of step (b) on one or more given target attributes of said object.

51. A method of monitoring the thermal impact of thermal processing on an object according to claim 50, characterized in that :

- in step (d), a sample of said at least one enzyme enclosed in said hermetically sealed container is obtained in the form of an enzyme solution by solubilizing in or more solvents the fraction of said solid dehydrated mix comprising said at least one enzyme, and
- in step (e) said enzyme solution is put into contact with a substrate for said at least one enzyme, resulting in a product, and measuring the residual activity of said at least one enzyme is effected by quantifying the rate of formation of said product.

52. A method of monitoring the thermal impact of thermal processing on an object according to claim 50 or claim 51, characterized in that said given target attribute of said object being quantified in step (f) is a chemical, physical, organoleptic or microbiological property of said object.

53. A method of monitoring the thermal impact of thermal processing on an object according to any of claims 50 to 52, wherein said object is human or animal food.

54. A method of monitoring the thermal impact of thermal processing on an object according to any of claims 50 to 52, wherein said object is a medical tool or device.

55. A method of monitoring the thermal impact of thermal processing on an object according to any of claims 50 to 52, wherein said object is a pharmaceutical composition in the form of a liquid, syrup, cream or paste.

56. A method of monitoring the thermal impact of thermal processing on an object according to any of claims 50 to 55, wherein said thermal processing is part of a pasteurization or sterilization process.

57. A method of monitoring the thermal impact of thermal processing on an object according to any of claims 50 to 56, wherein said at least one enzyme represents between 0.001 and 10% by weight, of said solid dehydrated mix.